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<p>(21) International Application Number: PCT/BG96/00013</p> <p>(22) International Filing Date: 22 October 1996 (22.10.96)</p> <p>(30) Priority Data: 100197 6 December 1995 (06.12.95) BG 100316 29 January 1996 (29.01.96) BG </p> <p>(71) Applicant (<i>for all designated States except US</i>): ANTIBIOTIC CO. [BG/BG]; 68 Aprilsko vastanie Boulevard, 7200 Razgrad (BG).</p> <p>(72) Inventors; and (75) Inventors/Applicants (<i>for US only</i>): DIMOV, Dimcho Ivanov [BG/BG]; 39 Knyaz Boris, 7200 Razgrad (BG). GROZ-DANOV, Georgy Asenov [BG/BG]; 36 Knyaz Boris Street, 7200 Razgrad (BG). PETKOV, Nedelcho Genov [BG/BG]; 17 Vaptsarov Street, 7200 Razgrad (BG). TODOROVA, Dimitra Tsoneva [BG/BG]; 4 Kiril y Metody Street, 7200 Razgrad (BG). DIMITROVA, Albena Stefanova [BG/BG]; 6 Tsigularska Street, 7200 Razgrad (BG).</p>		<p>(81) Designated States: AL, AU, BB, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: METHOD OF PRODUCTION OF LOVASTATIN</p> <p>(57) Abstract</p> <p>The method finds application in the pharmaceutical industry. Lovastatin is derived by this method from culture broth by filtration at values of pH 9.5÷13.0, included in a solid mass it is precipitated from the filtrate obtained, pH 2.5÷4.0, in the presence of an inert filler, antioxidant and a non-miscible with water organic solvent. It is extracted and lactonized in the medium of a chlorine-containing organic solvent. The latter is concentrated, and the residue is dissolved in a mixture of solvents having different polarity. After cooling at -10÷-30 °C, lovastatin is crystallized, dried and several times recrystallized.</p>			

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METHOD OF PRODUCTION OF LOVASTATIN

Field of technics

The invention is related to a method of production of lovastatin finding application in the pharmaceutical industry.

Preceding state of technics

Methods of isolation of lovastatin are known where the filtered culture broth, containing lovastatin, is extracted using ethyl acetate, the extract obtained is lactonized by boiling in toluene, and the isolated product is purified by chromatographing on silica gel (US 4444784, US 4450171).

Another method is known in which the culture broth of lovastatin is treated twice, pH 8.5÷9.0, filtered, extracted or purified by ultrafiltration/reverse osmosis, then it is recrystallized in ethyl acetate, butyl acetate or ethanol (BG 60460A).

A disadvantage of these methods is the necessity for using large volumes of extracting agents which leads to burdening expenses including also the ones for their reclamation with the view of protecting the environmental elements. The purification by extraction or by ultrafiltration/reverse osmosis demands usage of sophisticated and expensive equipment, the process are multistage, which contributes to the decrease of the yield. As a result of oxidation by-products are formed in the process of isolation of lovastatin and during the storage of the finished product obtained by any of the known methods. This leads to a decrease of the purity and the stability of the product.

The goal of the invention is creation of a method for production of lovastatin of high purity and stability, which is simplified, reliable, and employs small quantities of solvents.

Technical nature of the invention

The goal of the invention is achieved by a method, in which the culture broth containing lovastatin is treated with an alkaline base in the presence of an antioxidant, pH 9.5÷13.0, the mycelium is filtered and washed with a diluted solution of an alkaline base. The solution obtained is acidified with a mineral acid to pH 2.5÷4.0 in the presence of an antioxidant, inert filler and a 0.1÷0.3 % non-miscible with water organic solvent. The precipitate produced is filtered, dehydrated, extracted and lactonized by boiling in the medium of a chlorine-

containing organic solvent. The latter is distilled to a high concentration of the residue and dissolved in a mixture of acetonitrile-tert.-butylmethylether-butyl chloride in the presence of an antioxidant, and after cooling to $-10\text{--}30^\circ\text{C}$ is crystallized lovastatin, which is isolated and dried.

The filtered and dried raw lovastatin is dissolved in a nitrile such as acetonitrile or propionitrile. The solution obtained is decoloured using a mixture of activated carbon and aluminium oxide. After addition of water, lovastatin is precipitated at room temperature from the filtrate obtained. The filtrate is separated and the lovastatin obtained is recrystallized twice, successively in a mixture of acetate of a low alcohol-alkane C₇-C₉ at a temperature of $0\text{--}20^\circ\text{C}$ and then in acetone at a temperature of $-10\text{--}30^\circ\text{C}$. The crystalline product obtained is filtered and dried at a temperature of 50°C , and at an underpressure of 1 kPa. 102

As antioxidants are used : butylhydroxtoluene, butylhydroxyanisole, nordihydroguaiaretic acid, propyl ester of gallic acid, 2,2-methyleno-bis(6-tert.-butyl-4methyl-phenol), sodium pyrosulphite, sodium bisulphite, d,L- α -tocopherylacetate, hydroquinone, etc.

Silica gel, activated carbon, diatomite, kieselguhr, perlite, sawdust, wood shavings, zeolite are used as fillers for the precipitation of lovastatin.

Esters of aliphatic acids (amyl acetate, ethyl propionate, butyl formate, butyl acetate, etc.), chlorine-containing carbon hydrides (methylene chloride, chloroform, trichloroethylene, trichloroethane, perchloroethylene, dichloroethane, etc.) are used as non-miscible with water organic solvent.

Methyl acetate, ethyl acetate, propyl acetate or butyl acetate can be used as acetates of low alcohols, as the system of propyl acetate-isooctane is recommended.

The crystallization of lovastatin is performed in a mixture of solvents of different polarity in a suitable ratio, as mixtures of isopropanol-acetone, acetonitrile-butyl chloride, acetonitrile-tert.-butylmethylether-butyl chloride are used.

Advantages of the method according to the invention are production of lovastatin of high purity and yield, stable during storage, by using a simplified technological scheme and small quantities of solvents.

Examples for performance of the invention

To culture broth, containing 792 g of lovastatin (calculated as lactone), are added 16.3 g of butylhydroxytoluene in 165 ml of ethyl alcohol, the mixture is alkalified with a 40 % sodium hydroxide to pH 11.5 and stirred for 3 hours. The mycelium is filtered, washed six-fold - each time with 100 l of a 0.05 % sodium hydroxide. To the solution obtained are added 20 g of butylhydroxytoluene and 10 kg of perlite, and the mixture is acidified with a 20 % nitric acid to pH 2.7. After stirring for 30 min, 18 l of chloroform are added, and the mixture is stirred for another 1 h. The residue is separated from the filter-press. The product obtained is suspended in 100 l of chloroform and boiled in order to be to dehydrated using a metal Florence flask type of vessel until lovastatin acid is lactonized thoroughly. The process is monitored by HPLC. The chloroform solution is distilled to the consistence of oil which is dissolved in a mixture of 0.5 l of acetonitrile, 0.5 l of tert.-butylmethylether and 3.0 l of butyl chloride. After cooling to -20°C for 48 h the product crystallizes.

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After filtration and drying, the raw lovastatin is dissolved in 10 l of propionitrile at 35°C, decoloured using 280 g of activated carbon and 280 g aluminium oxide, and after filtration it is precipitated with 46 l of water at room temperature for 2 h. The filtered product is dissolved at 70°C in 3 l of propyl acetate, and after addition of 4 l of isooctane it is cooled to -20°C for 30 h. The filtered product is recrystallized in 2.5 l of acetone at a final temperature of -30°C, filtered and dried at a temperature of 50 °C and at an underpressure of 1 kPa.

601 g of lovastatin of 99.5 % purity are produced (HPLC).

Yield of lovastatin from culture broth - 75.5 %.

Optical rotation - -330° (c=5.0 mg/ml, CH₃CN).

IR and NMR spectra of the product are identical to these of Lovastatin USP Reference Standard.

PATENT CLAIMS

1. Method of production of lovastatin characterized by that, that lovastatin is derived from culture broth at a pH value of 9.5÷13.0, the mycelium is filtered, washed with a diluted alkaline base, and included in a solid mass lovastatin is precipitated in the filtrate, pH 2.5÷4.0, in the presence of an inert filler, antioxidant and a 0.1÷3.0 % non-miscible with water organic solvent, the precipitate is filtered, dehydrated azeotropically and lactonized by boiling in a medium of a chlorine-containing organic solvent, then the solvent is distilled to a high concentration of the residue, dissolved in a mixture of solvents of different polarity, the solution produced is cooled to -10÷-30°C, and the crystallized lovastatin is isolated, dried and recrystallized successively in a mixture of nitrile-water, by decolouring with aluminium oxide and activated carbon, and in a mixture of acetate of a low alcohol-alcane C₇-C₉, at a temperature of 0÷-20°C and in acetone at -10÷-30°C.
2. Method according to **Claim 1**, characterized by that, that butylhydroxytoluene, butylhydroxyanisole, nordihydroguaiaretic acid, propyl ester of gallic acid, 2,2-methylene-bis-(6-tert.-butyl-4-methyl-phenol), sodium pyrosulphite, sodium bisulphite, d,l- α -tocopheryl acetate, hydroquinone can be used as antioxidants.
3. Method according to **Claim 1** characterized by that, that methylene chloride, chloroform, trichloroethylene, trichloroethane, perchloroethylene, dichloroethane can be used as a chlorine-containing solvent for extraction and lactonization.
4. Method according to **Claim 1** and **Claim 2** characterized by that, that the following mixtures of solvents : Isopropanol-acetone, Acetonitrile-butyl chloride, Acetonitrile-tert.-butylmethylether-butyl chloride, Propyl acetate-isooctane, Ethyl acetate-n-heptane, can be used for dissolution and crystallization.

INTERNATIONAL SEARCH REPORT

Serial Application No

PCT/BG 96/00013

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D309/30 C12P17/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,94 10328 (BIOGAL GYOGYSZERGYAR) 11 May 1994 see page 1 - page 13 ---	1
Y	EP,A,0 033 538 (MERCK) 12 August 1981 cited in the application see page 4 - page 15 ---	1
A	WO,A,94 29292 (KRKA) 22 December 1994 see page 1 - page 6 -----	1

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Patent family members are listed in annex.

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Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9410328	11-05-94	HU-B-	210867	30-10-95
		AT-B-	401060	25-06-96
		AT-A-	901593	15-10-95
		CA-A-	2127381	11-05-94
		DE-T-	4395515	01-12-94
		ES-A-	2081776	01-03-96
		GR-A-	93100408	29-07-94
<hr/>				
EP-A-33538	12-08-81	US-A-	4293496	06-10-81
		AU-B-	548996	09-01-86
		AU-A-	6657381	13-08-81
		CA-A-	1199322	14-01-86
		CY-A-	1404	22-04-88
		HK-A-	16488	11-03-88
		JP-B-	1001476	11-01-89
		JP-C-	1528833	15-11-89
		JP-A-	56122375	25-09-81
		KE-A-	3746	02-10-87
		SU-A-	1318162	15-06-87
		US-A-	4444784	24-04-84
<hr/>				
WO-A-9429292	22-12-94	SI-A-	9300303	31-12-94
		BG-A-	100204	28-06-96
		CA-A-	2164411	22-12-94
		CZ-A-	9503251	17-04-96
		EP-A-	0702679	27-03-96
		HU-A-	72836	28-05-96
		PL-A-	311880	18-03-96
		SK-A-	148095	03-07-96
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